



Chakkarapani, E., Chau, V., Poskitt, K. J., Synnes, A., Kwan, E., Roland, E., & Miller, S. P. (2016). Low plasma magnesium is associated with impaired brain metabolism in neonates with hypoxic-ischaemic encephalopathy. *Acta Paediatrica*, 105(9), 1067-1073.
<https://doi.org/10.1111/apa.13505>

Peer reviewed version

Link to published version (if available):
[10.1111/apa.13505](https://doi.org/10.1111/apa.13505)

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Low plasma magnesium is associated with impaired brain metabolism in neonates with hypoxic-ischaemic encephalopathy.

Journal:	<i>Acta Paediatrica</i>
Manuscript ID	SPAE-2016-0129.R2
Manuscript Type:	Regular Article
Date Submitted by the Author:	n/a
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Keywords:	hypoxic ischemic encephalopathy, brain metabolism, therapeutic hypothermia, magnetic resonance spectroscopy, magnesium

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Title: Low plasma magnesium is associated with impaired brain metabolism in neonates with hypoxic-ischaemic encephalopathy.

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Short title: Hypomagnesaemia and brain metabolism

Abstract

Aim To determine the association between lowest plasma magnesium concentration and brain metabolism, and whether MRI brain injury patterns moderated the association in hypoxic-ischemic encephalopathy.

Methods In 131 early (day-of-life 3) and 65 late (day-of-life 10) scans of term encephalopathic infants born between 2004-2012, we examined the association of lowest plasma magnesium (until day-of-life 3) on basal-ganglia and white matter peak metabolite ratios on magnetic resonance spectroscopy independent of covariates and stratified by predominant patterns of injury [normal, basal-nuclei/total, watershed, multifocal] using multiple linear regression.

Results

Lowest plasma magnesium **was associated with** lower white matter N-acetyl-aspartate/Choline in the multifocal pattern on early-scan (regression-coefficient, β : 0.13; 95%CI: 0.04, 0.22) and in the basal-nuclei/total pattern on late-scan (β : 0.08; 95%CI: 0.02, 0.15), and was negatively associated with basal-ganglia lactate/N-acetyl-aspartate (β : -0.16; 95%CI: -0.05, -0.28)) and lactate/Choline (β : -0.1; 95%CI: -0.03, -0.17)) ratio in the basal-nuclei/total pattern on late scan independent of hypomagnesaemia correction, cooling and postmenstrual age at scan. Lowest plasma magnesium **was not associated** with metabolite ratios in other brain injury patterns.

Conclusion

In infants with hypoxic-ischaemic encephalopathy, predominant patterns of brain injury moderated the association between lowest plasma magnesium in the first 3 days of life and impaired brain metabolism.

Keywords: Newborn, hypoxic-ischaemic encephalopathy, plasma magnesium, brain metabolism, magnetic resonance spectroscopy

Keynotes

1. This retrospective cohort study assessed the association between low plasma magnesium concentration and brain metabolism stratified by the predominant patterns of brain injury in neonates with hypoxic-ischaemic encephalopathy.

2. We found that low plasma magnesium concentration was independently associated with adverse brain metabolism represented by higher lactate/N-acetyl-aspartate, higher lactate/choline ratios in the basal ganglia and lower N-acetyl-aspartate/choline ratios in the white matter.

3. The association between plasma magnesium concentration and brain metabolism is moderated by the predominant pattern of injury, seen only in neonates with the basal nuclei/total and multifocal patterns of MRI brain injury.

INTRODUCTION

Despite therapeutic hypothermia (TH), nearly 45% of cooled infants with hypoxic-ischaemic encephalopathy (HIE) experience adverse outcome of death or disability,(1) and infants with adverse outcome have impaired brain metabolism.(2) Brain metabolism is impaired by hypoxia-ischaemia due to disruption of mitochondrial metabolism and oxidative phosphorylation resulting in accumulation of lactic acid and reduction in N-acetyl aspartate (NAA: neuronal /axonal density and viability marker) relative to choline (Cho; membrane metabolism).(3) Changes in peak metabolite ratios including Lac/Cho(4) and Lac/NAA are predictive of long term neurodevelopment in infants with HIE.(2) Clinical factors contributing to impaired brain metabolism in infants with HIE are not fully understood.

Brain metabolism can be non-invasively assessed using proton magnetic resonance spectroscopy (5) and is crucial in providing energy to all cellular processes including ATP production, and for mitochondrial function.(6) Mitochondria are involved in the production of brain metabolites including NAA (7) and the functioning of mitochondria requires magnesium (Mg) for the synthesis of nucleic acids, proteins, and for intermediary metabolism.(8) Furthermore Mg may restore brain metabolism following hypoxic-ischaemic insult by blocking N-methyl D-aspartate receptor mediated calcium influx and stimulating sarcoplasmic reticulum mediated calcium sequestration thereby reducing calcium induced excitotoxic mitochondrial injury.(3, 8) Consequently hypomagnesaemia, which occurs commonly in infants with HIE(9) might adversely impact brain metabolism.

In the term neonate, hypoxia-ischaemia leads to a number of characteristic patterns of brain injury that reflect the severity and duration of the insult: basal nuclei predominant, watershed, total, and multifocal (stroke, white matter injury). (10) The predominant pattern of injury is significantly associated with peak metabolite ratios on the MRS. (11) Given this association, we hypothesized that low plasma Mg will be associated with impaired brain metabolism. Further we examined whether the pattern of brain injury moderated the relation between plasma Mg and brain metabolism.

PATIENTS AND METHODS

Human subjects

The University of British Columbia Clinical Research Ethics Board approved this study.

Inclusion criteria

The retrospective cohort included term newborns (>36 weeks gestation) admitted to the level III NICU at BC Women’s Hospital and Health Centre from June 2004 to December 2012, who met the following inclusion criteria: presence of clinically recognizable encephalopathy in the first day of life and at least one of the following: 1) foetal distress immediately preceding delivery, 2) requirement for resuscitation at birth, 3) Apgar score ≤ 5 at 5 min, or 4) metabolic acidosis (umbilical artery pH<7.1 or base deficit >10).

From 2008, infants with moderate or severe HIE or seizures(12) underwent TH within 6 hours of life, if they had one or more acute perinatal events (e.g. abruptio placenta) and indicators of peripartum hypoxia-ischaemia. Infants with metabolic or genetic syndromes or structural brain malformations were excluded. We included infants who underwent structural brain imaging along with MRS in basal ganglia and posterior white matter, and plasma Mg concentrations measured.

MR imaging

MRI brain was performed on day of life 3-6 (early) and on day of life 10-14 (late), as part of the local protocol of studying evolution of brain injuries in serial scans, using a specialized neonatal head coil (Sree Medical, Cleveland, Ohio, USA) on a Siemens (New Jersey, USA) 1.5 Tesla *Avanto* using VB 16 software and included the proton MRS. Proton MRS was acquired using single-voxel spectroscopy (TR: 1600; TE: 288; 150 averages) and the protocol remained same for all participants. The volume of interest (15x20x15mm) was placed on the right thalamus and on the occipito-parietal white matter ensuring no contamination occurred from the ventricles. Post-processing quantitative analysis was performed off-line on a Siemens workstation by the same study investigator. The four predominant patterns of lesions were categorized as normal, watershed, basal nuclei, total (maximal injury in the basal nuclei and watershed region), and multifocal (white

matter lesions and stroke).(13) As the basal nuclei predominant and total patterns of injury were significantly associated with more impaired brain metabolism compared with other patterns of injury (11) we grouped both patterns together.

Clinical data

Antenatal and perinatal factors were collected systematically from retrospective chart review. We calculated an encephalopathy score in the first 3 days of life, ranging from 0-7.(14) Plasma Mg concentration (mmol/L) and the lowest plasma Mg concentration during the first three days of life were collected. The reference range for plasma Mg concentration was 0.76-0.96 mmol/L. Hypomagnesaemia was defined as plasma Mg concentration below 0.76mmol/L. Data of Mg supplementation to correct hypomagnesaemia was collected including the dose and age of life.

Statistics

Normally distributed data were summarized as mean (SD) and data with skewed distribution were summarized as median (IQR). We used ANOVA and Kruskal Wallis test for more than 2 groups and Mann-Whitney U test for two groups, to identify the differences between normally distributed and non-symmetric distributed variables respectively. Chi-square test was used to detect difference in the proportion of cooled infants between the predominant patterns of brain injury.

Using multiple linear regression, we assessed the independent association of lowest plasma Mg concentration with the outcome variables: Lac/NAA, Lac/Cho and NAA/Cho obtained in the basal ganglia and white matter on early and late MRS accounting for covariates (i) TH, (ii) Mg therapy to correct hypomagnesaemia (yes/no) and (iii) postmenstrual age at MRS. To examine whether the relationship between plasma Mg concentration and brain metabolism differed by the pattern of injury we stratified the regression analyses by the predominant pattern of injury. Bivariate correlations between lowest plasma Mg concentration and individual metabolites were performed with spearman's rho. All statistical analyses were performed using IBM SPSS statistics v21.0 (North Harbour Portsmouth, Hampshire, England). Two sided P values <0.05 were considered significant.

RESULTS

Out of 223 infants with HIE, we had plasma Mg and early MRS/MRI data for 131 infants and late MRS/MRI data for 65 infants (70 early MRS/Mg; 61 both early & late MRS/Mg; 4 late MRS/Mg). There was no difference in the demographics, severity of asphyxia and encephalopathy between the infants included and excluded in the early or late MRS/Mg group. However the excluded infants were less likely to be cooled and had higher Apgar scores at 10 minutes compared to the included group. (Supplement Table 1). The demographic features of infants in the early and late scan group stratified by the predominant pattern of brain injury in the MRI are shown in Table 1. Of infants with multifocal predominant pattern of injury, 80% had white matter injury and 20% had stroke. Basal nuclei/Total pattern neonates had significantly higher Lac/NAA ratio compared to other patterns of injury. (Supplement Figure 1)

In the cohort, 89.3% of infants had hypomagnesaemia and 38.5% of these infants received Mg correction with a mean (SD) parenteral **Mg sulphate dose of 0.15mmol/kg (0.047)** after a median (IQR) duration of 3.2 hours (2.4, 6.0) of obtaining the blood sample that identified hypomagnesaemia. Infants with seizures were more likely to receive Mg correction than infants without seizures (79% versus 21%). Cooled and non-cooled infants did not differ in the incidence (94% vs 89%) and age mean (SD) (29.3h (17) versus 33.8h (19)) of hypomagnesaemia. Magnesium supplementation did not alter the relation between the predominant patterns of brain injury and the low plasma Mg concentration. (Supplemental Figure 2)

Association between plasma Mg concentration and peak metabolite ratios stratified by the predominant pattern of injury (Table 2)

Early MRS:

Basal nuclei and total pattern of injury

The lowest plasma Mg concentration was associated with white matter NAA/Cho in the univariate linear regression (0.08 (95% CI 0.005 to 0.15)) (Table 2), but was not significant when adjusted for covariates (TH, Mg therapy to correct hypomagnesaemia and

postmenstrual age at MRS). There was no significant association between lowest plasma Mg concentration and basal ganglia/white matter Lac/NAA ($P=0.08$) or Lac/Cho ($P=0.2$) ratios.

Multifocal pattern of injury:

Lower plasma Mg concentration was associated with lower white matter NAA/Cho ratio [0.13 (95% CI 0.04 to 0.22)] when adjusted for covariates. (Table 2) This relationship was supported by significant positive correlation between plasma Mg concentration and white matter NAA ($r=0.47$, $P=0.03$) and Cho ($r=0.51$, $P=0.02$).

Normal and watershed patterns of injury:

Low plasma Mg was not associated with the peak metabolite ratios.

Late MRS:

Basal nuclei and total pattern of injury

For every 0.1 mmol/L increase in the lowest plasma Mg concentration, Lac/NAA decreased by 0.16 (95% CI 0.05 to 0.28) and Lac/Cho decreased by 0.1 (95% CI 0.03 to 0.17) in the basal ganglia voxel and NAA/Cho increased by 0.08 (95%CI 0.02 to 0.15) in the white matter voxel when adjusted for covariates. (Table 2) The relationships were driven by higher lactate and lower NAA or Cho as indicated by weak negative correlation between increase in the lowest plasma Mg concentration and basal ganglia lactate ($r=-0.12$) and weak positive correlation between increase in the lowest plasma Mg concentration and basal ganglia NAA ($r=0.29$) and Cho ($r=0.26$).

Normal and watershed patterns of injury:

Low plasma Mg was not associated with the peak metabolite ratios.

The overlapping of infants in both early and late groups did not influence the relation between low plasma Mg and white matter NAA/Cho (early MRI multifocal pattern ($P=0.59$); late MRI basal nuclei/total pattern ($P=0.6$)); and basal ganglia Lac/NAA ($P=0.58$) and Lac/Cho ($P=0.48$) on late MRI basal nuclei/total pattern.

DISCUSSION

In term infants with hypoxic-ischemic encephalopathy, low plasma Mg within three days of age was associated with impaired brain metabolism assessed with MRS. This

association was moderated by the pattern of brain injury and was independent of undergoing therapeutic hypothermia, post menstrual age at MRS and receiving Mg to correct hypomagnesaemia. On early MRS, lower plasma Mg was associated with lower white matter NAA/Cho in multifocal predominant pattern of injury. On late MRS, lower plasma Mg concentration was significantly associated with higher basal ganglia Lac/NAA and Lac/Cho and lower white matter NAA/Cho in basal nuclei/total predominant pattern of injury.

Relationship between plasma Mg and brain metabolism differs by predominant pattern of brain injury

The relation between the low plasma Mg and brain metabolism can be confounded by the severity of encephalopathy and seizures. (9) The severity of encephalopathy and seizures relates to the predominant pattern of brain injury,(10) which is more objectively assessed at the same time as the MRS and in turn associated with brain metabolism.(11) Hence, we explored how the predominant pattern of injury moderated the relationship between plasma Mg concentration and the peak metabolite ratios.

We found that the predominant pattern of injury moderated the association of lowest plasma Mg concentration with brain oxidative metabolism: plasma Mg was associated with lactate, NAA and Cho levels only in neonates with the basal nuclei/total and multifocal patterns of injury. In our cohort, the multifocal pattern of injury comprised of predominantly white matter injury rather than stroke. Mg influences the activity of enzymes essential for the functioning of mitochondria, (8) which underpins brain metabolism. (15) Furthermore defective mitochondrial oxidative phosphorylation is associated with low intracellular brain Mg and improvement in mitochondrial oxidative phosphorylation is associated with increase in Mg levels.(16) The moderation effect by the basal nuclei/total and multifocal pattern of injury might be due to the abundance of mitochondria in the basal nuclei and white matter.(17) The preponderance of Mg responsive glutamate receptors and higher metabolic activity in the basal nuclei might be responsible for the relation between low plasma Mg and the lactate ratios in the basal nuclei/total pattern. (18)

Low plasma Mg concentration was correlated with lower NAA or Cho. N-acetylaspartate, which is synthesised in the mitochondria(19) is likely to be reduced secondary to mitochondrial dysfunction following hypoxia-ischaemia and low Mg. We observed the association between lactate ratios and Mg concentration only on the late MRS. This might be due to the secondary rise and persistence of cerebral lactate for a longer duration (20) and declining NAA or Cho, which might reflect neural injury and inflammatory reaction through microglia activation or astrocytosis. (21)

Serum Mg accounts for 0.3% of total body Mg and exists in ionized and protein bound form. There are conflicting reports of hypo and hypermagnesaemia following birth asphyxia. This variability is likely related to varied definitions of asphyxia and the presence of acidosis that may give a falsely elevated value. (22) The age of lowest plasma Mg concentration in our study was beyond 20 hours, when the acidosis had normalised. We did not observe an association between the correction of hypomagnesaemia and brain metabolism. This may be due to the inherent delay in the diagnosis and inadequate treatment of hypomagnesaemia in clinical practice. As the anti-excitotoxicity effect of Mg is likely to be within few hours of age, a therapeutic window might have been missed.

The relation between plasma and brain Mg is not clearly understood. Brain intracellular Mg increases during hypoxia-ischaemia(23) followed by elevation of plasma Mg,(24) which could be an innate neuroprotective mechanism. Low plasma Mg might reflect the exhaustion of the innate neuroprotective effect related to the severity of hypoxic-ischaemic insult and might reflect impaired brain metabolism. Furthermore, brain intracellular Mg appears to be tightly controlled, as infusion of Magnesium sulphate during hypoxic-ischaemic insult only briefly elevated the intracellular Mg and possibly worsened brain metabolites.(23) Consequently Mg therapy may not necessarily improve brain metabolism.

There are limitations to our study. Although the majority of the excluded infants due to missing data were not cooled, we had similar proportion of cooling in the infants included in the study across different patterns of brain injury. Although 61 infants had both early and

late MRS, the overlapping of infants in both groups did not influence any of the associations between plasma Mg and metabolite ratios. The small sample size precluded analysing the effect of time (year of recruitment) on the observed relationships and might have reduced the power in the subgroup analysis. We measured plasma Mg and treated hypomagnesaemia on a clinical basis resulting in sicker infants being included in the study. Consequently it is unknown if low plasma Mg will affect brain metabolism in the excluded less sick infants. There was no data of albumin, total parenteral nutrition use, total duration of low Mg and the Mg concentration at the time of MRS (3-6 or 10-14day-of-life). However as excitotoxicity occurs within hours of a hypoxic-ischemic insult affecting brain metabolism, we studied plasma Mg within 3 days of life. All the infants had uniform MRI acquisition and the neuroradiologist blinded to the clinical information assessed the pattern of brain injury, reducing bias in our outcome measure. All spectra were visually inspected for quality; automated assessment of artefacts such as motion, were not used. We do not have the long term outcome of these infants to assess the significance of the association between the lowest plasma Mg concentration and peak metabolite ratios in relation to long term outcome.

Therapeutic hypothermia is currently the standard of care for infants with HIE. The higher rate of hypomagnesaemia in the cooled infants and the association between hypomagnesaemia and brain metabolism stress the importance of better monitoring of plasma Mg concentration. These data suggest the hypothesis that avoiding hypomagnesaemia might contribute to improving brain metabolism.

Conclusion

In term infants with hypoxic-ischemic encephalopathy low plasma Mg concentration is associated with impaired brain metabolism independent of hypomagnesaemia correction, therapeutic hypothermia and postmenstrual age of undergoing MRS. This association is moderated by the predominant pattern of injury and is particularly evident in neonates with the basal nuclei & total and multifocal predominant patterns of injury.

Abbreviations

Cho Choline

HIE hypoxic-ischaemic encephalopathy

Lac Lactate

Mg Magnesium

MRI Magnetic resonance image

MRS Magnetic resonance spectroscopy

NAA N-acetyl aspartate

TH Therapeutic hypothermia

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Table 1: Baseline demographics in early and late MRS/Mg groups stratified by predominant pattern of brain injury.

Variable	Early MRS/Mg group				Late MRS/Mg group			
	Normal N=65	Watershed N=12	BN/Total N=28	Multifocal N=26	Normal N=24	Watershed N=8	BN/Total N=18	Multifocal N=15
Gestation weeks Mean (SD)	39.1 (1.53)	39.4 (1.24)	39.1 (1.41)	39.0 (1.36)	39.6 (1.47)	39.5 (1.41)	38.8(1.38)	38.5 (1.59)
Birth weight Kg Mean (SD)	3.3 (0.69)	3.3 (0.38)	3.5 (0.72)	3.3 (0.55)	3.5 (0.61)	3.3 (0.41)	3.3 (0.49)	3.3 (0.55)
Male (%)	60%	58%	57%	62%	58%	50%	44%	72%
Cord pH Mean (SD)	7.08 (0.18)	7.03 (0.13)	7.02 (0.18)	7.07 (0.14)	7.03 (0.19)	7.01 (0.13)	7.05 (0.17)	7.09 (0.12)
10 min Apgar Med (IQR)	6 (4,7)	7 (6,8)	4 (3,6)*	6 (7,9)	5 (4,7)	7 (5,7)	4 (3,7)	7 (5,7)
Encephalopathy score Median (IQR)	5 (5,7)	7 (6,7)**	7 (7,7)**	6 (4,7)**	6 (5,7)	7 (6,7)	7 (6,7)	7 (5,7)
Cooled infants (%)	37%	33%	43%	12%	67%	50%	56%	33%
Lowest plasma Mg mmol/L Mean (SD)	0.65 (0.09)	0.65(0.06)	0.60(0.09)	0.65(0.10)	0.66(0.07)	0.67(0.06)	0.62(0.1)	0.63(0.1)
Age of low plasma Mg hours Median (IQR)	23.5 (11.4, 33.8)	38.8 (21.2, 57.4)	22.0 (14.9, 28.3)	24.8 (15.8, 36.9)	35.3 [‡] (22.1, 53.1)	39.3 [‡] (24.7, 57.3)	23.0 [‡] (19.9, 34.3)	23.5 [‡] (12.5, 31.5)
Mg treated Hypomagnesaemia infants (%)	30%	25%	62%	41%	5%	13%	65%	58%
Age Mg correction given Median (IQR)	20.3h (14.5,27.3)	21 h ¶	30.4h (27.4,52.5)	33.4h (15.0,66.0)	31.0h ¶	¶	29.4h (28.0, 54.0)	24.3h (14.8, 34.5)

* BN/total significantly different from Watershed and Multifocal groups. ** Watershed, BN&Total and Multifocal significantly different from normal pattern.

[‡] Significant difference between the patterns of injury. ¶ Missing data.

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Table 2: Regression coefficients for association between low plasma Mg and basal ganglia and white matter peak metabolite ratios in early and late MRS.

		Basal ganglia spectra		White matter spectra	
Patterns of brain injury	Peak metabolite ratios	Unadjusted coefficients (95% CI)	Adjusted coefficients (95% CI)*	Unadjusted coefficients (95% CI)	Adjusted coefficients (95% CI)*
Early MRS					
Basal nuclei & total	NAA/Cho			0.08 (0.005, 0.15)	
Multifocal	NAA/Cho			0.12 (0.04, 0.22)	0.13 (0.04, 0.22)
Late MRS					
Basal nuclei & total	Lac/NAA	-0.14 (-0.03, -0.25)	-0.16 (-0.05, -0.28)		
	Lac/Cho	-0.08 (-0.02, -0.13)	-0.1 (-0.03, -0.17)		
	NAA/Cho			0.08 (0.02, 0.15)	0.085 (0.02, 0.15)

* adjusted for therapeutic hypothermia (yes or no), Mg therapy to correct hypomagnesaemia (yes or no) and postmenstrual age at scan.

Title: Low plasma magnesium is associated with impaired brain metabolism in neonates with hypoxic-ischaemic encephalopathy.

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Short title: Hypomagnesaemia and brain metabolism

Abstract

Aim To determine the association between lowest plasma magnesium concentration and brain metabolism, and whether MRI brain injury patterns moderated the association in hypoxic-ischemic encephalopathy.

Methods In 131 early (day-of-life 3) and 65 late (day-of-life 10) scans of term encephalopathic infants born between 2004-2012, we examined the association of lowest plasma magnesium (until day-of-life 3) on basal-ganglia and white matter peak metabolite ratios on magnetic resonance spectroscopy independent of covariates and stratified by predominant patterns of injury [normal, basal-nuclei/total, watershed, multifocal] using multiple linear regression.

Results

Lowest plasma magnesium was associated with lower white matter N-acetyl-aspartate/Choline in the multifocal pattern on early-scan (regression-coefficient, β : 0.13; 95%CI: 0.04, 0.22) and in the basal-nuclei/total pattern on late-scan (β : 0.08; 95%CI: 0.02, 0.15), and was negatively associated with basal-ganglia lactate/N-acetyl-aspartate (β : -0.16; 95%CI: -0.05, -0.28)) and lactate/Choline (β : -0.1; 95%CI: -0.03, -0.17)) ratio in the basal-nuclei/total pattern on late scan independent of hypomagnesaemia correction, cooling and postmenstrual age at scan. Lowest plasma magnesium was not associated with metabolite ratios in other brain injury patterns.

Conclusion

In infants with hypoxic-ischaemic encephalopathy, predominant patterns of brain injury moderated the association between lowest plasma magnesium in the first 3 days of life and impaired brain metabolism.

Keywords: Newborn, hypoxic-ischaemic encephalopathy, plasma magnesium, brain metabolism, magnetic resonance spectroscopy

Keynotes

1. This retrospective cohort study assessed the association between low plasma magnesium concentration and brain metabolism stratified by the predominant patterns of brain injury in neonates with hypoxic-ischaemic encephalopathy.

2. We found that low plasma magnesium concentration was independently associated with adverse brain metabolism represented by higher lactate/N-acetyl-aspartate, higher lactate/choline ratios in the basal ganglia and lower N-acetyl-aspartate/choline ratios in the white matter.

3. The association between plasma magnesium concentration and brain metabolism is moderated by the predominant pattern of injury, seen only in neonates with the basal nuclei/total and multifocal patterns of MRI brain injury.

INTRODUCTION

Despite therapeutic hypothermia (TH), nearly 45% of cooled infants with hypoxic-ischaemic encephalopathy (HIE) experience adverse outcome of death or disability,(1) and infants with adverse outcome have impaired brain metabolism.(2) Brain metabolism is impaired by hypoxia-ischaemia due to disruption of mitochondrial metabolism and oxidative phosphorylation resulting in accumulation of lactic acid and reduction in N-acetyl aspartate (NAA: neuronal /axonal density and viability marker) relative to choline (Cho; membrane metabolism).(3) Changes in peak metabolite ratios including Lac/Cho(4) and Lac/NAA are predictive of long term neurodevelopment in infants with HIE.(2) Clinical factors contributing to impaired brain metabolism in infants with HIE are not fully understood.

Brain metabolism can be non-invasively assessed using proton magnetic resonance spectroscopy (5) and is crucial in providing energy to all cellular processes including ATP production, and for mitochondrial function.(6) Mitochondria are involved in the production of brain metabolites including NAA (7) and the functioning of mitochondria requires magnesium (Mg) for the synthesis of nucleic acids, proteins, and for intermediary metabolism.(8) Furthermore Mg may restore brain metabolism following hypoxic-ischaemic insult by blocking N-methyl D-aspartate receptor mediated calcium influx and stimulating sarcoplasmic reticulum mediated calcium sequestration thereby reducing calcium induced excitotoxic mitochondrial injury.(3, 8) Consequently hypomagnesaemia, which occurs commonly in infants with HIE(9) might adversely impact brain metabolism.

In the term neonate, hypoxia-ischaemia leads to a number of characteristic patterns of brain injury that reflect the severity and duration of the insult: basal nuclei predominant, watershed, total, and multifocal (stroke, white matter injury). (10) The predominant pattern of injury is significantly associated with peak metabolite ratios on the MRS. (11) Given this association, we hypothesized that low plasma Mg will be associated with impaired brain metabolism. Further we examined whether the pattern of brain injury moderated the relation between plasma Mg and brain metabolism.

PATIENTS AND METHODS

Human subjects

The University of British Columbia Clinical Research Ethics Board approved this study.

Inclusion criteria

The retrospective cohort included term newborns (>36 weeks gestation) admitted to the level III NICU at BC Women's Hospital and Health Centre from June 2004 to December 2012, who met the following inclusion criteria: presence of clinically recognizable encephalopathy in the first day of life and at least one of the following: 1) foetal distress immediately preceding delivery, 2) requirement for resuscitation at birth, 3) Apgar score ≤ 5 at 5 min, or 4) metabolic acidosis (umbilical artery pH<7.1 or base deficit >10).

From 2008, infants with moderate or severe HIE or seizures(12) underwent TH within 6 hours of life, if they had one or more acute perinatal events (e.g. abruptio placenta) and indicators of peripartum hypoxia-ischaemia. Infants with metabolic or genetic syndromes or structural brain malformations were excluded. We included infants who underwent structural brain imaging along with MRS in basal ganglia and posterior white matter, and plasma Mg concentrations measured.

MR imaging

MRI brain was performed on day of life 3-6 (early) and on day of life 10-14 (late), as part of the local protocol of studying evolution of brain injuries in serial scans, using a specialized neonatal head coil (Sree Medical, Cleveland, Ohio, USA) on a Siemens (New Jersey, USA) 1.5 Tesla *Avanto* using VB 16 software and included the proton MRS. Proton MRS was acquired using single-voxel spectroscopy (TR: 1600; TE: 288; 150 averages) and the protocol remained same for all participants. The volume of interest (15x20x15mm) was placed on the right thalamus and on the occipito-parietal white matter ensuring no contamination occurred from the ventricles. Post-processing quantitative analysis was performed off-line on a Siemens workstation by the same study investigator. The four predominant patterns of lesions were categorized as normal, watershed, basal nuclei, total (maximal injury in the basal nuclei and watershed region), and multifocal (white

matter lesions and stroke).(13) As the basal nuclei predominant and total patterns of injury were significantly associated with more impaired brain metabolism compared with other patterns of injury (11) we grouped both patterns together.

Clinical data

Antenatal and perinatal factors were collected systematically from retrospective chart review. We calculated an encephalopathy score in the first 3 days of life, ranging from 0-7.(14) Plasma Mg concentration (mmol/L) and the lowest plasma Mg concentration during the first three days of life were collected. The reference range for plasma Mg concentration was 0.76-0.96 mmol/L. Hypomagnesaemia was defined as plasma Mg concentration below 0.76mmol/L. Data of Mg supplementation to correct hypomagnesaemia was collected including the dose and age of life.

Statistics

Normally distributed data were summarized as mean (SD) and data with skewed distribution were summarized as median (IQR). We used ANOVA and Kruskal Wallis test for more than 2 groups and Mann-Whitney U test for two groups, to identify the differences between normally distributed and non-symmetric distributed variables respectively. Chi-square test was used to detect difference in the proportion of cooled infants between the predominant patterns of brain injury.

Using multiple linear regression, we assessed the independent association of lowest plasma Mg concentration with the outcome variables: Lac/NAA, Lac/Cho and NAA/Cho obtained in the basal ganglia and white matter on early and late MRS accounting for covariates (i) TH, (ii) Mg therapy to correct hypomagnesaemia (yes/no) and (iii) postmenstrual age at MRS. To examine whether the relationship between plasma Mg concentration and brain metabolism differed by the pattern of injury we stratified the regression analyses by the predominant pattern of injury. Bivariate correlations between lowest plasma Mg concentration and individual metabolites were performed with spearman’s rho. All statistical analyses were performed using IBM SPSS statistics v21.0 (North Harbour Portsmouth, Hampshire, England). Two sided P values <0.05 were considered significant.

RESULTS

Out of 223 infants with HIE, we had plasma Mg and early MRS/MRI data for 131 infants and late MRS/MRI data for 65 infants (70 early MRS/Mg; 61 both early & late MRS/Mg; 4 late MRS/Mg). There was no difference in the demographics, severity of asphyxia and encephalopathy between the infants included and excluded in the early or late MRS/Mg group. However the excluded infants were less likely to be cooled and had higher Apgar scores at 10 minutes compared to the included group. (Supplement Table 1). The demographic features of infants in the early and late scan group stratified by the predominant pattern of brain injury in the MRI are shown in Table 1. Of infants with multifocal predominant pattern of injury, 80% had white matter injury and 20% had stroke. Basal nuclei/Total pattern neonates had significantly higher Lac/NAA ratio compared to other patterns of injury. (Supplement Figure 1)

In the cohort, 89.3% of infants had hypomagnesaemia and 38.5% of these infants received Mg correction with a mean (SD) parenteral Mg sulphate dose of 0.15mmol/kg (0.047) after a median (IQR) duration of 3.2 hours (2.4, 6.0) of obtaining the blood sample that identified hypomagnesaemia. Infants with seizures were more likely to receive Mg correction than infants without seizures (79% versus 21%). Cooled and non-cooled infants did not differ in the incidence (94% vs 89%) and age mean (SD) (29.3h (17) versus 33.8h (19)) of hypomagnesaemia. Magnesium supplementation did not alter the relation between the predominant patterns of brain injury and the low plasma Mg concentration. (Supplemental Figure 2)

Association between plasma Mg concentration and peak metabolite ratios stratified by the predominant pattern of injury (Table 2)

Early MRS:

Basal nuclei and total pattern of injury

The lowest plasma Mg concentration was associated with white matter NAA/Cho in the univariate linear regression (0.08 (95% CI 0.005 to 0.15)) (Table 2), but was not significant when adjusted for covariates (TH, Mg therapy to correct hypomagnesaemia and

postmenstrual age at MRS). There was no significant association between lowest plasma Mg concentration and basal ganglia/white matter Lac/NAA (P=0.08) or Lac/Cho (P=0.2) ratios.

Multifocal pattern of injury:

Lower plasma Mg concentration was associated with lower white matter NAA/Cho ratio [0.13 (95% CI 0.04 to 0.22)] when adjusted for covariates. (Table 2) This relationship was supported by significant positive correlation between plasma Mg concentration and white matter NAA (r=0.47, P=0.03) and Cho (r=0.51, P=0.02).

Normal and watershed patterns of injury:

Low plasma Mg was not associated with the peak metabolite ratios.

Late MRS:

Basal nuclei and total pattern of injury

For every 0.1 mmol/L increase in the lowest plasma Mg concentration, Lac/NAA decreased by 0.16 (95% CI 0.05 to 0.28) and Lac/Cho decreased by 0.1 (95% CI 0.03 to 0.17) in the basal ganglia voxel and NAA/Cho increased by 0.08 (95%CI 0.02 to 0.15) in the white matter voxel when adjusted for covariates. (Table 2) The relationships were driven by higher lactate and lower NAA or Cho as indicated by weak negative correlation between increase in the lowest plasma Mg concentration and basal ganglia lactate (r=-0.12) and weak positive correlation between increase in the lowest plasma Mg concentration and basal ganglia NAA (r=0.29) and Cho (r=0.26).

Normal and watershed patterns of injury:

Low plasma Mg was not associated with the peak metabolite ratios.

The overlapping of infants in both early and late groups did not influence the relation between low plasma Mg and white matter NAA/Cho (early MRI multifocal pattern (P=0.59); late MRI basal nuclei/total pattern (P=0.6)); and basal ganglia Lac/NAA (P=0.58) and Lac/Cho (P=0.48) on late MRI basal nuclei/total pattern.

DISCUSSION

In term infants with hypoxic-ischemic encephalopathy, low plasma Mg within three days of age was associated with impaired brain metabolism assessed with MRS. This

association was moderated by the pattern of brain injury and was independent of undergoing therapeutic hypothermia, post menstrual age at MRS and receiving Mg to correct hypomagnesaemia. On early MRS, lower plasma Mg was associated with lower white matter NAA/Cho in multifocal predominant pattern of injury. On late MRS, lower plasma Mg concentration was significantly associated with higher basal ganglia Lac/NAA and Lac/Cho and lower white matter NAA/Cho in basal nuclei/total predominant pattern of injury.

Relationship between plasma Mg and brain metabolism differs by predominant pattern of brain injury

The relation between the low plasma Mg and brain metabolism can be confounded by the severity of encephalopathy and seizures. (9) The severity of encephalopathy and seizures relates to the predominant pattern of brain injury,(10) which is more objectively assessed at the same time as the MRS and in turn associated with brain metabolism.(11) Hence, we explored how the predominant pattern of injury moderated the relationship between plasma Mg concentration and the peak metabolite ratios.

We found that the predominant pattern of injury moderated the association of lowest plasma Mg concentration with brain oxidative metabolism: plasma Mg was associated with lactate, NAA and Cho levels only in neonates with the basal nuclei/total and multifocal patterns of injury. In our cohort, the multifocal pattern of injury comprised of predominantly white matter injury rather than stroke. Mg influences the activity of enzymes essential for the functioning of mitochondria, (8) which underpins brain metabolism. (15) Furthermore defective mitochondrial oxidative phosphorylation is associated with low intracellular brain Mg and improvement in mitochondrial oxidative phosphorylation is associated with increase in Mg levels.(16) The moderation effect by the basal nuclei/total and multifocal pattern of injury might be due to the abundance of mitochondria in the basal nuclei and white matter.(17) The preponderance of Mg responsive glutamate receptors and higher metabolic activity in the basal nuclei might be responsible for the relation between low plasma Mg and the lactate ratios in the basal nuclei/total pattern. (18)

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Low plasma Mg concentration was correlated with lower NAA or Cho. N-acetylaspartate, which is synthesised in the mitochondria(19) is likely to be reduced secondary to mitochondrial dysfunction following hypoxia-ischaemia and low Mg. We observed the association between lactate ratios and Mg concentration only on the late MRS. This might be due to the secondary rise and persistence of cerebral lactate for a longer duration (20) and declining NAA or Cho, which might reflect neural injury and inflammatory reaction through microglia activation or astrocytosis. (21)

Serum Mg accounts for 0.3% of total body Mg and exists in ionized and protein bound form. There are conflicting reports of hypo and hypermagnesaemia following birth asphyxia. This variability is likely related to varied definitions of asphyxia and the presence of acidosis that may give a falsely elevated value. (22) The age of lowest plasma Mg concentration in our study was beyond 20 hours, when the acidosis had normalised. We did not observe an association between the correction of hypomagnesaemia and brain metabolism. This may be due to the inherent delay in the diagnosis and inadequate treatment of hypomagnesaemia in clinical practice. As the anti-excitotoxicity effect of Mg is likely to be within few hours of age, a therapeutic window might have been missed.

The relation between plasma and brain Mg is not clearly understood. Brain intracellular Mg increases during hypoxia-ischaemia(23) followed by elevation of plasma Mg,(24) which could be an innate neuroprotective mechanism. Low plasma Mg might reflect the exhaustion of the innate neuroprotective effect related to the severity of hypoxic-ischaemic insult and might reflect impaired brain metabolism. Furthermore, brain intracellular Mg appears to be tightly controlled, as infusion of Magnesium sulphate during hypoxic-ischaemic insult only briefly elevated the intracellular Mg and possibly worsened brain metabolites.(23) Consequently Mg therapy may not necessarily improve brain metabolism.

There are limitations to our study. Although the majority of the excluded infants due to missing data were not cooled, we had similar proportion of cooling in the infants included in the study across different patterns of brain injury. Although 61 infants had both early and

late MRS, the overlapping of infants in both groups did not influence any of the associations between plasma Mg and metabolite ratios. The small sample size precluded analysing the effect of time (year of recruitment) on the observed relationships and might have reduced the power in the subgroup analysis. We measured plasma Mg and treated hypomagnesaemia on a clinical basis resulting in sicker infants being included in the study. Consequently it is unknown if low plasma Mg will affect brain metabolism in the excluded less sick infants. There was no data of albumin, total parenteral nutrition use, total duration of low Mg and the Mg concentration at the time of MRS (3-6 or 10-14day-of-life). However as excitotoxicity occurs within hours of a hypoxic-ischemic insult affecting brain metabolism, we studied plasma Mg within 3 days of life. All the infants had uniform MRI acquisition and the neuroradiologist blinded to the clinical information assessed the pattern of brain injury, reducing bias in our outcome measure. All spectra were visually inspected for quality; automated assessment of artefacts such as motion, were not used. We do not have the long term outcome of these infants to assess the significance of the association between the lowest plasma Mg concentration and peak metabolite ratios in relation to long term outcome.

Therapeutic hypothermia is currently the standard of care for infants with HIE. The higher rate of hypomagnesaemia in the cooled infants and the association between hypomagnesaemia and brain metabolism stress the importance of better monitoring of plasma Mg concentration. These data suggest the hypothesis that avoiding hypomagnesaemia might contribute to improving brain metabolism.

Conclusion

In term infants with hypoxic-ischemic encephalopathy low plasma Mg concentration is associated with impaired brain metabolism independent of hypomagnesaemia correction, therapeutic hypothermia and postmenstrual age of undergoing MRS. This association is moderated by the predominant pattern of injury and is particularly evident in neonates with the basal nuclei & total and multifocal predominant patterns of injury.

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Abbreviations

- Cho Choline
- HIE hypoxic-ischaemic encephalopathy
- Lac Lactate
- Mg Magnesium
- MRI Magnetic resonance image
- MRS Magnetic resonance spectroscopy
- NAA N-acetyl aspartate
- TH Therapeutic hypothermia

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Table 1: Baseline demographics in early and late MRS/Mg groups stratified by predominant pattern of brain injury.

Variable	Early MRS/Mg group				Late MRS/Mg group			
	Normal N=65	Watershed N=12	BN/Total N=28	Multifocal N=26	Normal N=24	Watershed N=8	BN/Total N=18	Multifocal N=15
Gestation weeks Mean (SD)	39.1 (1.53)	39.4 (1.24)	39.1 (1.41)	39.0 (1.36)	39.6 (1.47)	39.5 (1.41)	38.8(1.38)	38.5 (1.59)
Birth weight Kg Mean (SD)	3.3 (0.69)	3.3 (0.38)	3.5 (0.72)	3.3 (0.55)	3.5 (0.61)	3.3 (0.41)	3.3 (0.49)	3.3 (0.55)
Male (%)	60%	58%	57%	62%	58%	50%	44%	72%
Cord pH Mean (SD)	7.08 (0.18)	7.03 (0.13)	7.02 (0.18)	7.07 (0.14)	7.03 (0.19)	7.01 (0.13)	7.05 (0.17)	7.09 (0.12)
10 min Apgar Med (IQR)	6 (4,7)	7 (6,8)	4 (3,6)*	6 (7,9)	5 (4,7)	7 (5,7)	4 (3,7)	7 (5,7)
Encephalopathy score Median (IQR)	5 (5,7)	7 (6,7)**	7 (7,7)**	6 (4,7)**	6 (5,7)	7 (6,7)	7 (6,7)	7 (5,7)
Cooled infants (%)	37%	33%	43%	12%	67%	50%	56%	33%
Lowest plasma Mg mmol/L Mean (SD)	0.65 (0.09)	0.65(0.06)	0.60(0.09)	0.65(0.10)	0.66(0.07)	0.67(0.06)	0.62(0.1)	0.63(0.1)
Age of low plasma Mg hours Median (IQR)	23.5 (11.4, 33.8)	38.8 (21.2, 57.4)	22.0 (14.9, 28.3)	24.8 (15.8, 36.9)	35.3 ‡ (22.1, 53.1)	39.3 ‡ (24.7, 57.3)	23.0 ‡ (19.9, 34.3)	23.5 ‡ (12.5, 31.5)
Mg treated Hypomagnesaemia infants (%)	30%	25%	62%	41%	5%	13%	65%	58%
Age Mg correction given Median (IQR)	20.3h (14.5,27.3)	21 h ¶	30.4h (27.4,52.5)	33.4h (15.0,66.0)	31.0h ¶	¶	29.4h (28.0, 54.0)	24.3h (14.8, 34.5)

* BN/total significantly different from Watershed and Multifocal groups. ** Watershed, BN&Total and Multifocal significantly different from normal pattern.

‡ Significant difference between the patterns of injury. ¶ Missing data.

Table 2: Regression coefficients for association between low plasma Mg and basal ganglia and white matter peak metabolite ratios in early and late MRS.

		Basal ganglia spectra		White matter spectra	
Patterns of brain injury	Peak metabolite ratios	Unadjusted coefficients (95% CI)	Adjusted coefficients (95% CI)*	Unadjusted coefficients (95% CI)	Adjusted coefficients (95% CI)*
Early MRS					
Basal nuclei & total	NAA/Cho			0.08 (0.005, 0.15)	
Multifocal	NAA/Cho			0.12 (0.04, 0.22)	0.13 (0.04, 0.22)
Late MRS					
Basal nuclei & total	Lac/NAA	-0.14 (-0.03, -0.25)	-0.16 (-0.05, -0.28)		
	Lac/Cho	-0.08 (-0.02, -0.13)	-0.1 (-0.03, -0.17)		
	NAA/Cho			0.08 (0.02, 0.15)	0.085 (0.02, 0.15)

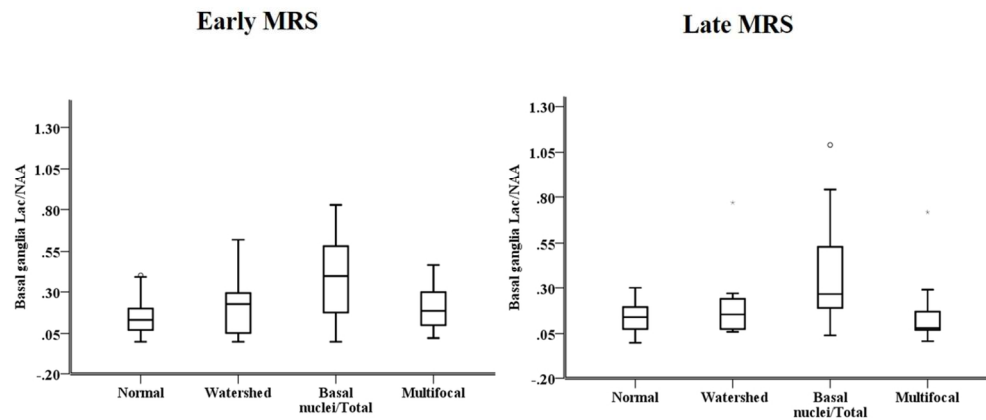
* adjusted for therapeutic hypothermia (yes or no), Mg therapy to correct hypomagnesaemia (yes or no) and postmenstrual age at scan.

Supplemental table 1

Table S-1: Baseline characteristics of infants included and excluded in the early and late MRS/Mg group. Out of 223 infants in the cohort, the early MRI/MRS and plasma Mg concentration group comprised of 131 infants (missing data, MRS: 23; Mg data: 64, both MRS and Mg: 5).

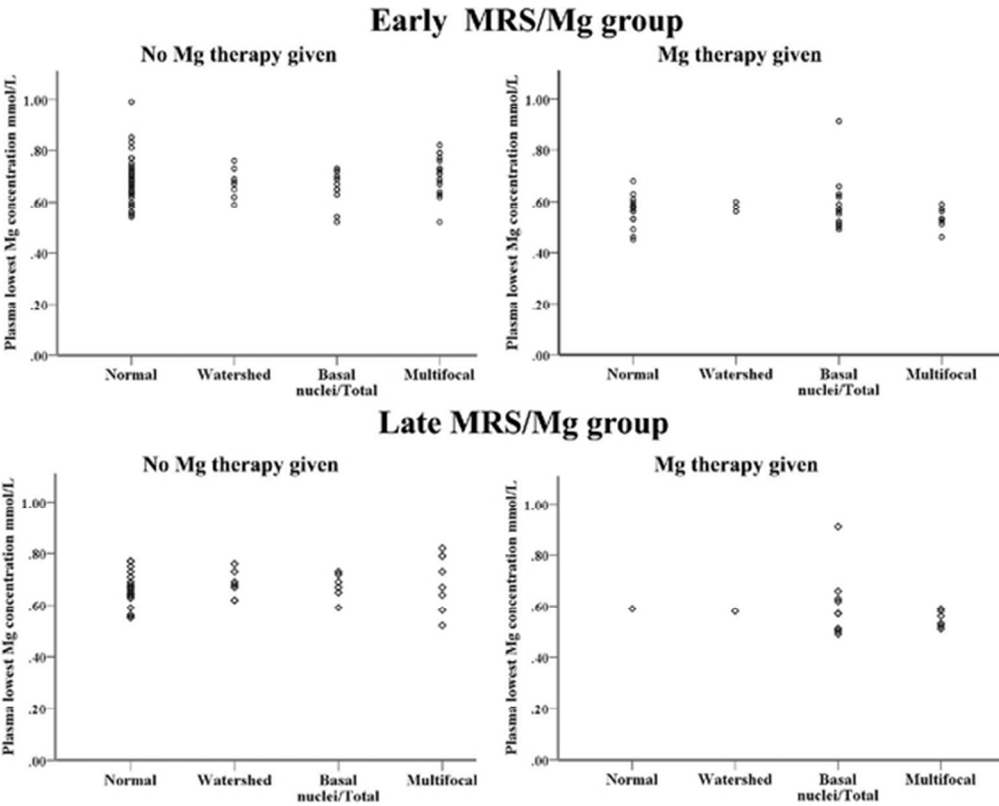
- $P \leq 0.05$

Variable	Early MRS/Mg group		Late MRS/Mg group	
	Included in the study N=131	Excluded in the study due to missing data. N=92	Included in the study N=65	Excluded in the study due to missing data. N=158
Gestation weeks				
Mean (SD)	39.1 (1.43)	39.3 (1.76)	39.1 (1.50)	39.2 (1.61)
Birth weight Kg				
Mean (SD)	3.34 (0.65)	3.3 (0.57)	3.3 (0.64)	3.4 (0.55)
Head circumference cm				
Mean (SD)	34.6 (2.57)	34.4 (1.63)	34.7 (1.69)	34.4 (2.40)
Male (%)	60%	55%	57%	58%
Cord pH				
Mean (SD)	7.06 (0.17)	7.02 (0.19)	7.05 (0.16)	7.04 (0.19)
10 min Apgar				
Med (IQR)	6 (4,7)	7 (5,9)	6 (4,7)*	7 (4,8)*
Encephalopathy score				
Median (IQR)	7 (5,7)	6 (5,7)	7 (5,7)	6 (5,7)
Cooled infants (%)	33%	21%*	54%	17%*



Supplement Figure 1: Fig 1: Distribution of basal ganglia Lac/NAA ratios in early and late MRS/Mg groups based on the predominant patterns of brain injury.
* ° outliers.

114x51mm (300 x 300 DPI)



Supplement Figure 2: Plasma lowest magnesium concentration in different patterns of brain injury on early and late scans based on magnesium therapy for hypomagnesaemia.
52x41mm (300 x 300 DPI)